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Supplementary Information for

Exercise preserves physical fitness during aging through AMPK and mitochondrial dynamics

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Supplementary Materials and Methods

Healthspan parameters

Crawling distance was measured in solid plates as previously described (1) with minor modifications. Briefly, we transferred worms to unseeded plates and recorded a 30-seconds video at a rate of 15 frames per second using a stereomicroscope (Optika SMZ-4) coupled with a device camera. Recorded images were analyzed using the ImageJ plugin Worm-tracker (wrMTrck) (2). At least 15 animals were recorded per biological replicate.

Pharyngeal pumping rates were measured by counting grinder contraction in the terminal bulb in 30-seconds intervals. At least 10 animals were evaluated per biological replicate.

Heat stress experiments were performed by exposing worms to 35°C for 9 hours (3). Briefly, we transferred worms to pre-heated plates seeded with OP50-1 and scored for live versus dead. At least 15 animals were recorded per biological replicate.

Lifespan

Lifespan experiments were performed as described previously (4). Briefly, worms were passaged at 20°C for at least two generations before lifespan assays were initiated. Synchronized populations of L1 animals were obtained by hypochlorite treatment, then allowed to develop at 20°C on standard NGM plates seeded with OP50-1. Adult animals (day 1) were then exposed to control or acute exercise protocol, and 100-150 worms were transferred to fresh NGM agar plates with 40 worms per plate. Survival was scored every day and a worm was deemed dead when unresponsive to tap on the head and tail. All lifespan results shown in figure panels are representative of three independent experiments, with detailed information presented in Table S3.

Imaging

Activation of mitochondrial unfolded protein response (mtURP) was assessed using the whole-body reporters *hsp-6p*::GFP and *hsp-60p*::GFP as described elsewhere (5). Strains expressing fluorescent proteins specifically in the body wall muscle were used to evaluate autophagy (P*myo-3*::*lgg*-1::CFP) and mitochondrial network morphology [(P*myo-3*::omYFP + P*myo-3*::mls::CFP) and (P*myo-3*::mitoGFP (matrix GFP) + pRF4)]. Briefly, worms were anesthetized in Tetramisole 0.2 mg.ml^{-1}, mounted on 2% agarose pads on glass slides, and subsequently imaged on Zeiss Axio Imager M2 fluorescence microscope with Axiocam HRC camera.

Proteomics Analysis

For analysis processed proteomics data was utilized. For the PCA plots the normalized individual protein expression levels were used and plotted using R version 4.0.3 and R program ggplot2 version 3.3.5 (https://ggplot2.tidyverse.org/index.html). Heatmaps were displayed using R program pheatmap version 1.0.12 (Kolde, R. (2015). pheatmap: Pretty heatmaps [Software]). Volcano plots were made use R program EnhancedVolcano (Blighe et al., 2018). Ortholog human protein names were determined from the *C. elegans* symbols by utilizing WormBase database (6). To associate genes to human mitochondrial gene and pathways, we overlayed the Human MitoCarta 3.0 genes (7) with the log2(fold-change) values from the protein expression for each comparison. The Human MitoCarta database has the associated mitochondrial pathways associated with each gene and categorized in three levels of hierarchy. The first level has the seven main mitochondrial pathway categories, the second level has 39, and the third level has 103. In the heatmaps, we displayed the first and second level pathways associated with each gene and grouped the genes accordingly. We displayed all the mitochondrial related proteins for all MitoCarta genes in each pathway that were present in our data set. We designated if the comparison for each protein was significance (i.e., adjusted p-value < 0.05) for each protein with a *.

Quantitative RT-PCR (qRT-PCR)

For each condition, approximately 250 animals were collected. Total RNA was extracted using TRIzol (Sigma-Aldrich, St. Louis, MO). First-strand cDNA was synthesized from each sample using M-MLV reverse transcriptase kit (Sigma-Aldrich, St. Louis, MO). SYBR green was used to perform qRT-PCR (ABI 7500). At least two biological replicates were examined for each condition. *drp-1* and *hlh-1* gene expression fold changes were calculated using the ΔΔCt method and normalized to the housekeeping gene *act-1*. Primer sequences: *drp-1* (F- CGGAGAAAGAGCGGTCAGTGC / R- TGTGGGGACGTCTTGCTGCT), *hlh-1* (F- GAGTCCACGTGTCACCGCAA / R- TGGAGCATAGGACGGGAGGT), *act-1* (F- CTACGAACTTCCTGACGGACAAG / R- CCGGCGGACTCCATACC).

Plate dietary restriction assay

Solid plate-based dietary restriction assays were performed as described elsewhere (8). Briefly, *Ad libitum* and dietary restriction plates were prepared with a bacterial concentration of 10^{11} cfu/ml and 10^{8} cfu/ml bacterial concentration, respectively. FUdR was added on top of the bacterial lawn (1mg/ml) 24 hours before worms were introduced to the plates on the first day of adulthood. Worms were kept under either Ad libitum or dietary restriction for 10 days, then physical fitness was assessed.

Western Blotting

Approximately 500 animals were collected and sonicated in RIPA buffer. Lysates were centrifuged at 10000 g, 10 min, 4°C to remove debris before measuring protein concentration using the Bradford assay. Laemmli buffer was added before protein samples were boiled 95°C for 10 min. Western blot analysis was performed under standard conditions with antibodies against phospho-AMPKα Thr172 (Cell signaling #2535, 1:1000); β-actin (Santa Cruz sc-4778, 1:500).

O2 consumption

Oxygen consumption rates were measured using high-resolution respirometry (Oroboros Oxygraph). 200 animals (day 1) were collected from seeded NGM plates and incubated with FUdR (20 mg.ml^{-1}) or vehicle for 4 hours in M9 buffer. Oxygen consumption was measured in M9 buffer for 15 min at 20°C. The slopes were used to calculate oxygen consumption rates. We performed 3 independent experiments with 3 technical replicates each.

Food ingestion during exercise

We estimated *C. elegans* food ingestion during the exercise protocol period by counting the number of colony-forming units in the M9 buffer after 4 hours of swimming and compared with mock condition (no worms). Briefly, seeded NGM agar plates were flooded with M9 buffer. A total of 500 animals was transferred to the plates and allowed to swim. After 4 hours of swimming, M9 buffer containing worms (or mock condition) was collected, centrifuged at 700 rpm for 1 min, and the number of CFUs was assessed in the worm free supernatant fraction. Bacteria concentration was determined through serial dilution, plating, and counting of CFUs. After subtracting the mock condition (blank) from the exercise condition, we estimated that each animal ingested a total of 20k bacteria or ~90 bacteria/min during the exercise protocol (see figure on the side). For this measurement, we performed 3 independent experiments with 5 technical replicates each. This amount of food ingestion by worms in liquid environment is within the range of maximal growth rates for *C. elegans* (between 10^4 and 10^5 CFUs per nematode per day), as described elsewhere (9).

Supplementary Figures

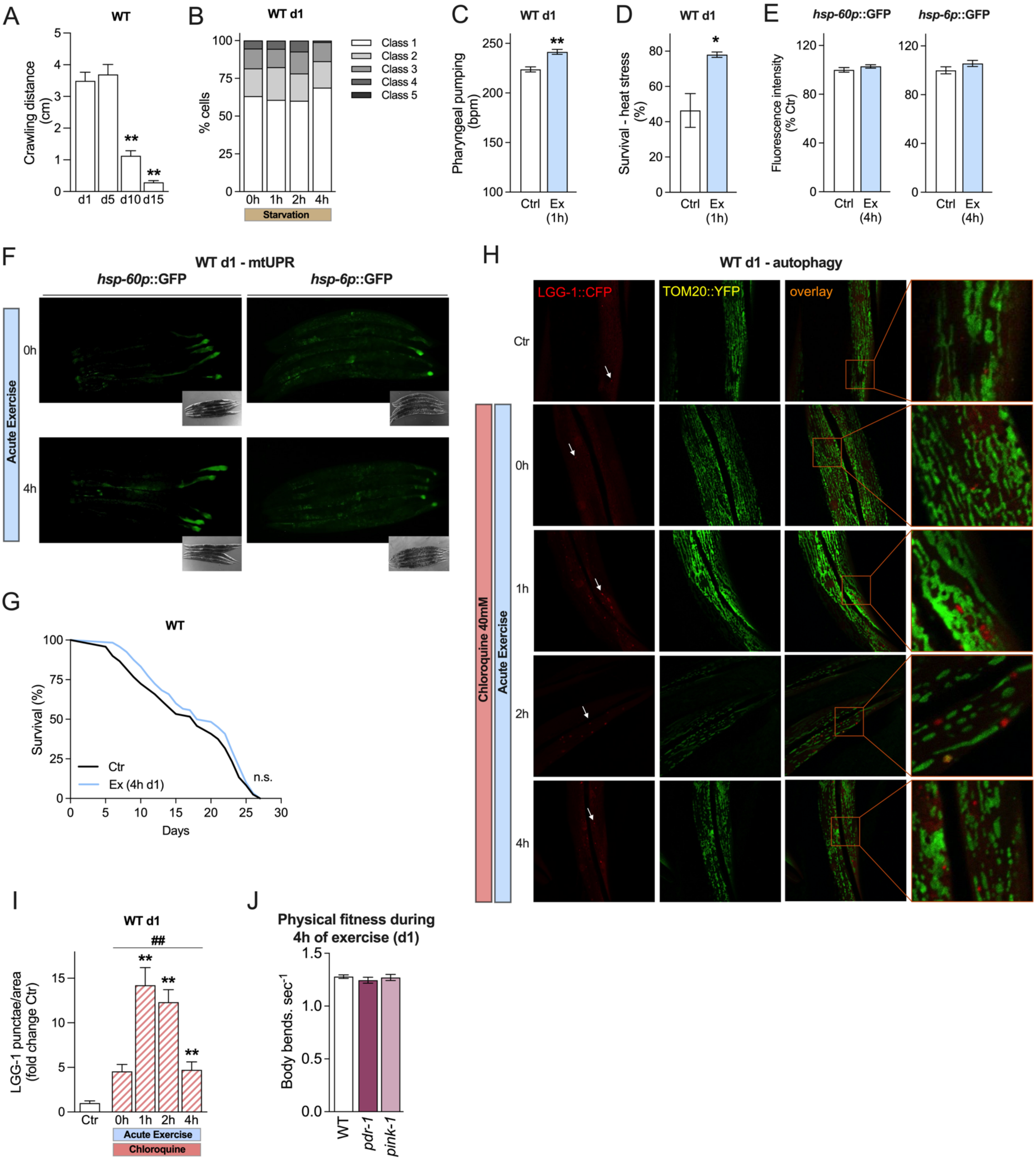


Fig. S1. Short-term exercise enhances healthspan parameters and increases autophagic flux (related to Fig. 1). (A) Decay in crawling distance in WT worms during aging. (B) Mitochondrial morphology in body wall muscle cells of WT worms subjected to starvation. (C) Pharyngeal pumping and (D) survival - heat stress (35°C for 9 hours) in WT worms submitted to 1 hour of exercise on day 1 of adulthood. (E) Quantification and (F) representative images of mitochondrial unfolded protein response (mtUPR) activity (5) in worms submitted to 4 hours of exercise on day 1 of adulthood. (G) Lifespan of WT worms submitted to 1 hour of exercise on day 1 of adulthood. (H, I) Representative images and quantification of muscle LGG-1::CFP punctae (white arrows) in animals pre-treated with Chloroquine (40mM) for 4 hours and submitted to 4 hours of exercise on day 1 of adulthood. Chloroquine was used to measure the autophagic flux through inhibition of lysosomal proteolysis, as described elsewhere (10). (J) Physical fitness during 4 hours of acute exercise (average of 0h, 1h, 2h and 4h) on day 1 of adulthood of WT and mitophagy mutants *pdr-1(gk488)* and *pink-1(tm17790)*. Data are presented as mean ± SEM. *p <0.05 and **p <0.001 vs. d1 or Ctr. Detailed statistical analyses, number of biological replicates and sample size are described in *SI Appendix*, Table S3.

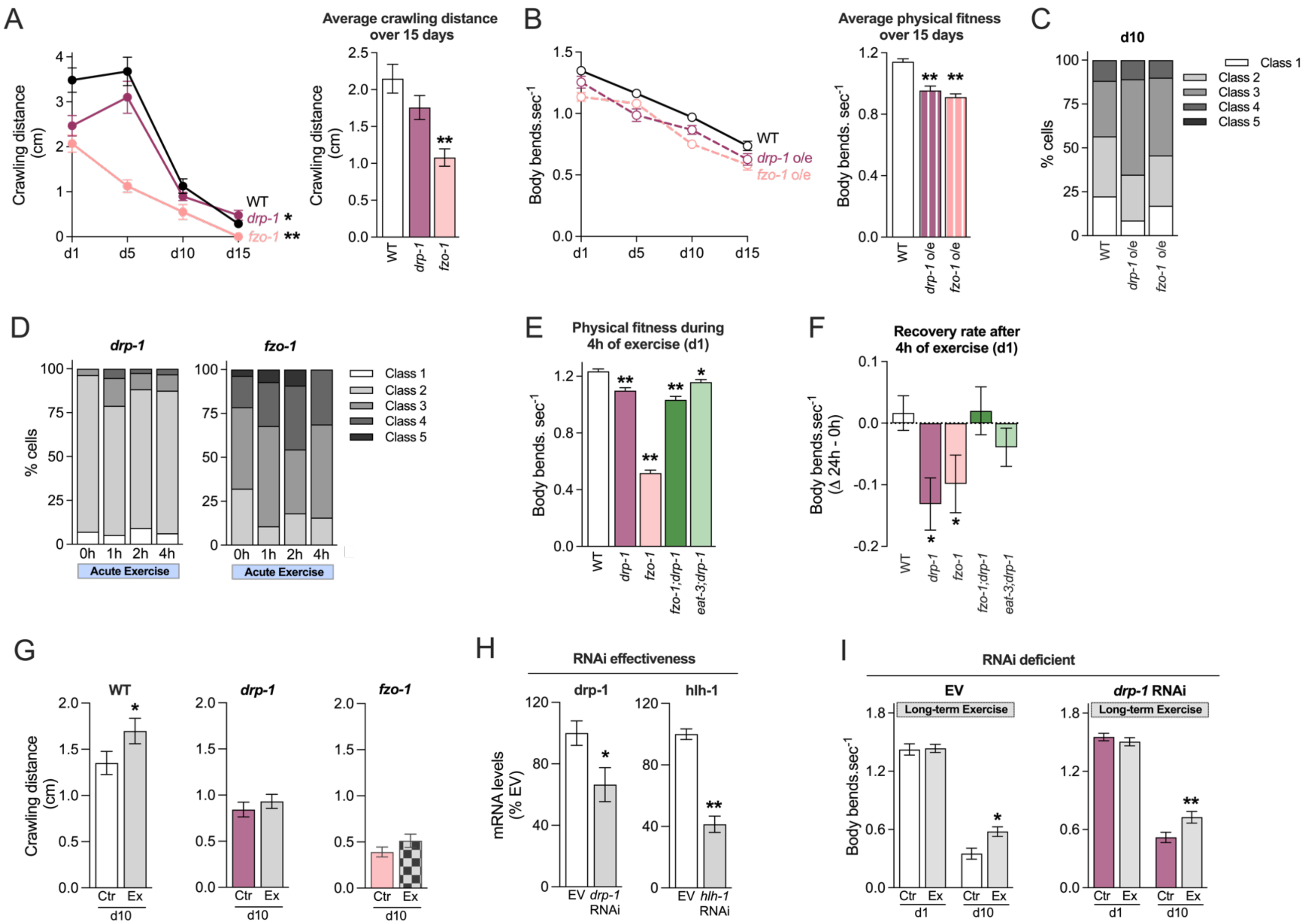


Fig. S2. Impaired mitochondrial fission or fusion compromises physical fitness (related to Fig. 2). (A) Crawling distance decay (left panel) and average crawling distance (right panel, average of d1, d5, d10 and d15) of WT worms and mitochondrial dynamics mutants *drp-1(tm1108)* and *fzo-1(tm1133)* during aging. (B) Physical fitness decay (left panel) and average physical fitness (right panel, average of d1, d5, d10 and d15) and (C) mitochondrial morphology in body wall muscle cells of WT compared to worms overexpressing (o/e) the mitochondrial dynamics genes *drp-1* and *fzo-1*. (D) Mitochondrial morphology in body wall muscle cells of the mitochondrial dynamics mutants *drp-1(tm1108)* and *fzo-1(tm1133)* submitted to acute exercise on day 1 of adulthood. (E) Physical fitness during 4 hours of exercise (average of 0h, 1h, 2h and 4h) and (F) recovery rate of WT and mitochondrial dynamics mutants *drp-1(tm1108), fzo-1(tm1133), fzo-1(tm1133);drp-1(tm1108)* and *eat-3(ad426);drp-1(tm1108)* submitted to acute exercise on day 1 of adulthood. (G) Crawling distance of WT and mitochondrial dynamics mutants *drp-1(tm1108), fzo-1(tm1133), fzo-1(tm1133);drp-1(tm1108)* and *eat-3(ad426);drp-1(tm1108)* submitted to long-term exercise. (H) mRNA levels of muscle-specific RNAi strain *sid-1(qt-9);myo-3p::sid-1* fed empty vector (EV) control or *drp-1* and *hlh-1* RNAi for 72 hours from L4 stage. (I) Physical fitness of RNAi deficient strain *sid-1(qt-9)* fed empty vector (EV) control *or drp-1* RNAi from L4 stage, and submitted to long-term exercise. Data are presented as mean ± SEM. *p <0.05 and **p <0.001 vs. WT, Ctr or EV. Detailed statistical analyses, number of biological replicates and sample size are described in *SI Appendix*, Table S3.

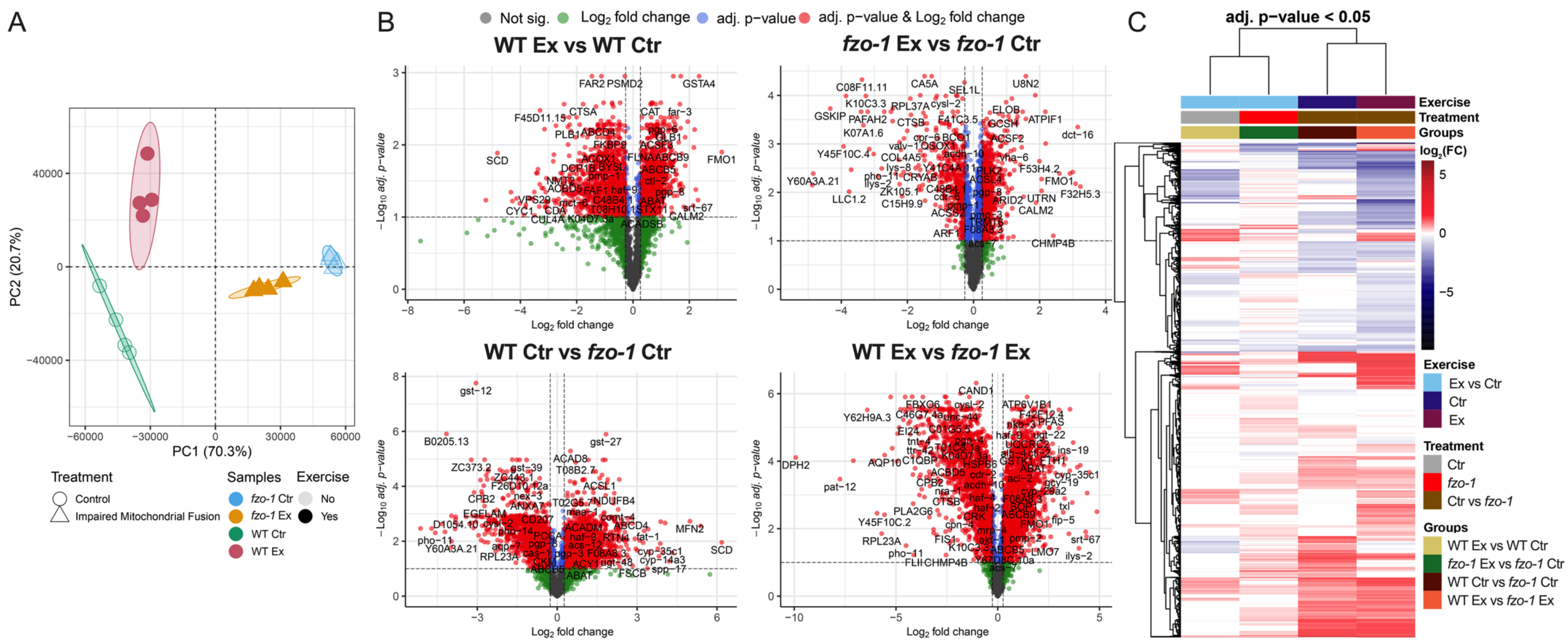


Fig. S3. Global pattern of protein changes comparing control (WT) and *fzo-1(tm1133)* worms submitted to long-term exercise (related to Fig. 3). (A) Principal component analysis (PCA) plot on the overall protein expression for all proteins in the data set. (B) Volcano plots for each comparison with adjusted p-value for the significance. The red colored dots are proteins which have both an adjusted p-value cutoff < 0.05 and absolute value for fold-change < 1.2. (C) Heatmap for the log2(Fold-Change) values for each comparison. The top annotation bars label each column to designate the group name, exercise condition, and treatment. Hierarchical clustering was used to order the proteins (n=4 per group).

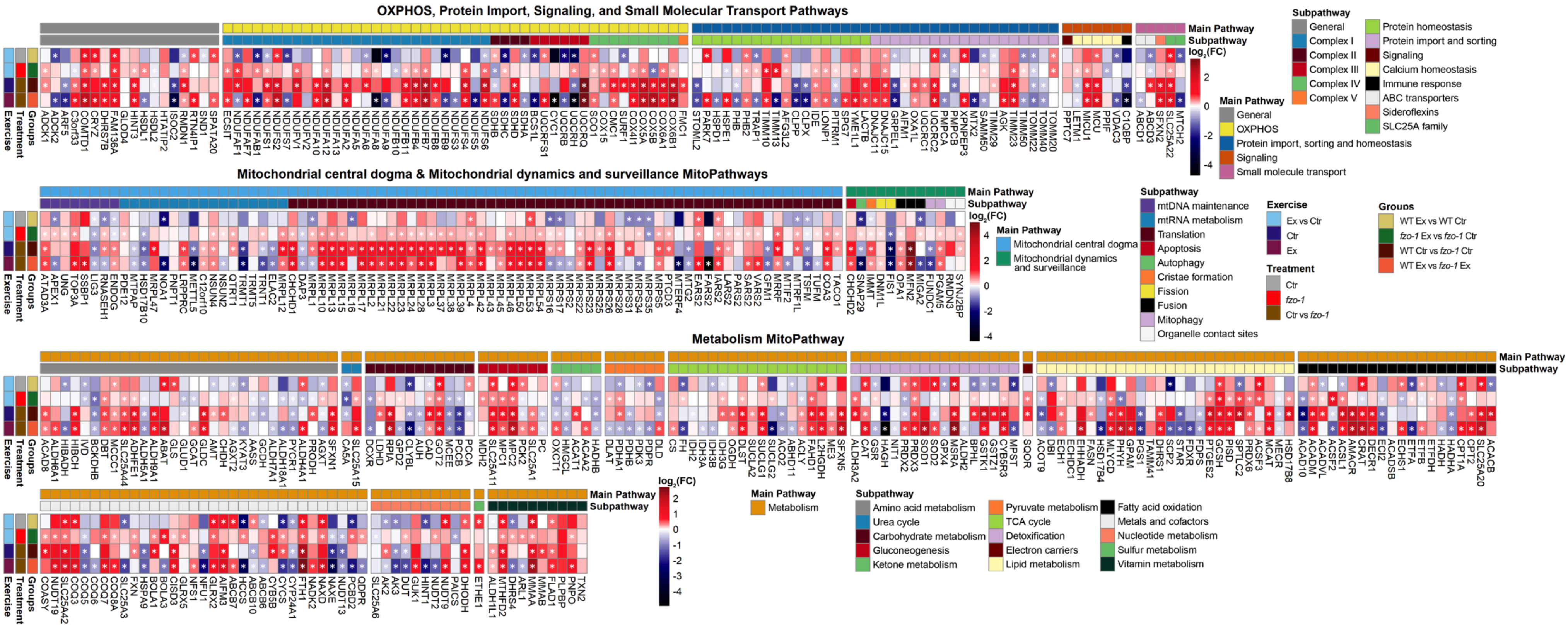
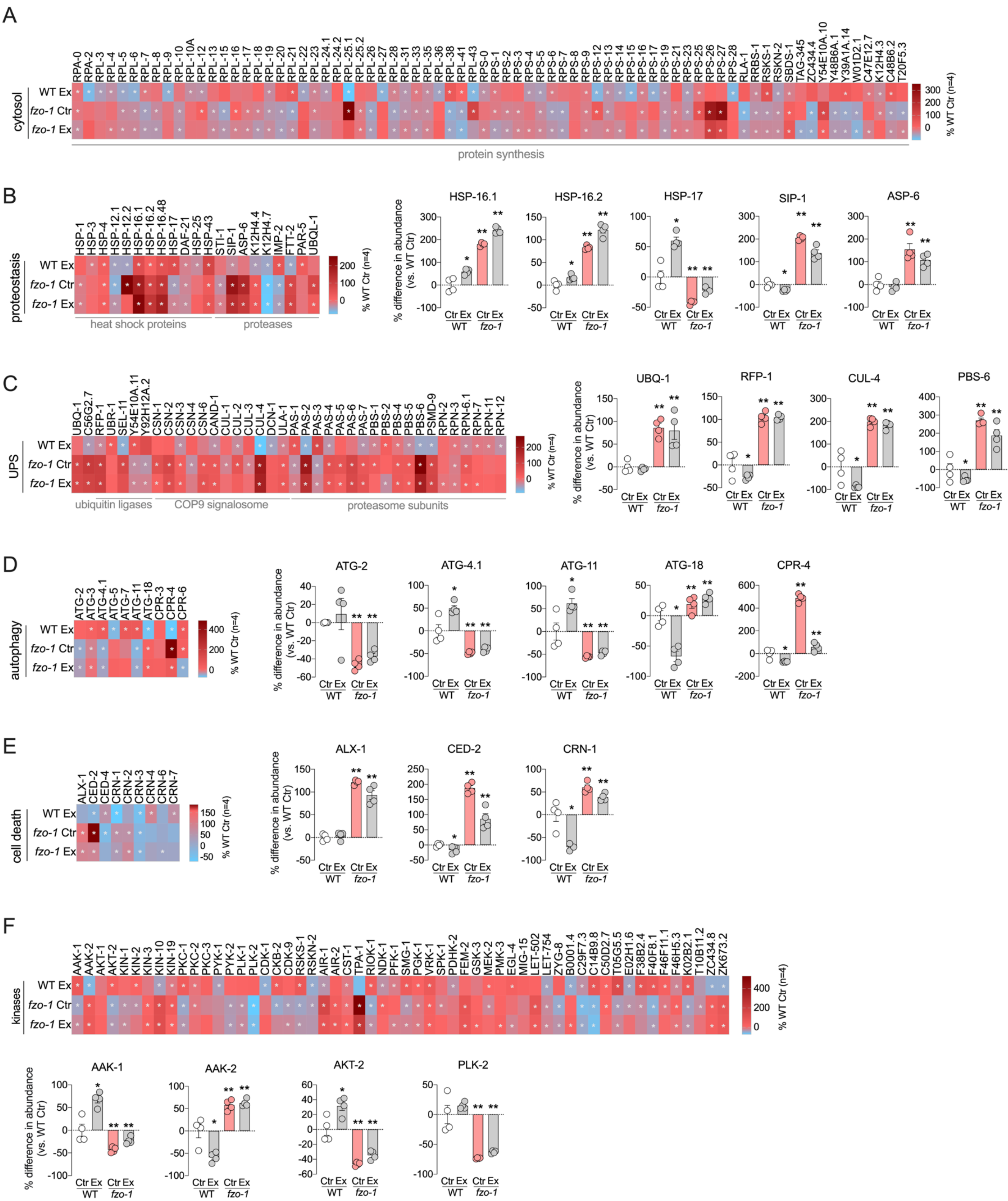


Fig. S4. Mitochondrial protein changes comparing control (WT) and *fzo-1(tm1133)* worms submitted to long-term exercise (related to Fig. 3). Heatmaps displaying log2(Fold-Change) values for each comparison with the * showing if the that protein for that comparison has an adjusted p-value < 0.05. The specific proteins being displayed are the overlapping mitochondrial proteins from the MitoCarta gene list. The top annotation bars label each column to designate the specific mitochondrial pathways that each protein is associated with. The row annotation bars label designate the group name, exercise condition, and treatment (n=4 per group). Changes in protein abundance were determined by comparison between WT and *fzo-1(tm1133)* worms that were or were not submitted to long-term exercise [starting at the onset of adulthood (day 1), according to the protocol described in Figure 2D]. Proteomics was performed at day 10.



A
RPA-0 RPA-2 RPL-3 RPL-4 RPL-5 RPL-6 RPL-7 RPL-8 RPL-9 RPL-10 RPL-10A RPL-12 RPL-13 RPL-15 RPL-16 RPL-17 RPL-18 RPL-19 RPL-20 RPL-21 RPL-22 RPL-23 RPL-24.1 RPL-24.2 RPL-25.1 RPL-25.2 RPL-26 RPL-27 RPL-28 RPL-31 RPL-33 RPL-35 RPL-36 RPL-38 RPL-41 RPL-43 RPS-0 RPS-1 RPS-2 RPS-3 RPS-4 RPS-5 RPS-6 RPS-7 RPS-8 RPS-9 RPS-12 RPS-13 RPS-14 RPS-15 RPS-16 RPS-17 RPS-19 RPS-21 RPS-23 RPS-25 RPS-26 RPS-27 RPS-28 RLA-1 RRBS-1 RSKS-1 RSKN-2 SBDS-1 TAG-345 ZC434.4 Y54E10A.10 Y48B6A.1 Y39A1A.14 W01D2.1 C47E12.7 K12H4.3 C48B6.2 T20F5.3
cytosol
WT Ex
fzo-1 Ctr
fzo-1 Ex
protein synthesis
300 200 100 0
% WT Ctr (n=4)
B
HSP-1 HSP-3 HSP-4 HSP-12.1 HSP-12.2 HSP-16.1 HSP-16.2 HSP-16.48 HSP-17 DAF-21 HSP-25 HSP-43 STI-1 SIP-1 ASP-6 K12H4.4 K12H4.7 IMP-2 FTT-2 PAR-5 UBQL-1
proteostasis
heat shock proteins
proteases
200 100 0
% WT Ctr (n=4)
HSP-16.1
HSP-16.2
HSP-17
SIP-1
ASP-6
% difference in abundance (vs. WT Ctr)
Ctr Ex
WT
fzo-1
C
UBQ-1 C56G2.7 RFP-1 UBR-1 SEL-11 Y54E10A.11 Y92H12A.2 CSN-1 CSN-2 CSN-3 CSN-4 CSN-6 CAND-1 CUL-1 CUL-2 CUL-3 CUL-4 DCN-1 ULA-1 PAS-1 PAS-2 PAS-3 PAS-4 PAS-5 PAS-6 PAS-7 PBS-1 PBS-2 PBS-4 PBS-5 PBS-6 PSMD-9 RPN-2 RPN-3 RPN-6.1 RPN-7 RPN-11 RPN-12
UPS
ubiquitin ligases
COP9 signalosome
proteasome subunits
UBQ-1
RFP-1
CUL-4
PBS-6
D
ATG-2 ATG-3 ATG-4.1 ATG-5 ATG-7 ATG-11 ATG-18 CPR-3 CPR-4 CPR-6
autophagy
400 200 0
ATG-2
ATG-4.1
ATG-11
ATG-18
CPR-4
E
ALX-1 CED-2 CED-4 CRN-1 CRN-2 CRN-3 CRN-4 CRN-6 CRN-7
cell death
150 100 50 0 -50
ALX-1
CED-2
CRN-1
F
AAK-1 AAK-2 AKT-1 AKT-2 KIN-1 KIN-2 KIN-3 KIN-10 KIN-19 PKC-1 PKC-2 PKC-3 PYK-1 PYK-2 PLK-1 PLK-2 CDK-1 CKB-2 CDK-9 RSKS-1 RSKN-2 AIR-1 AIR-2 CST-1 TPA-1 RIOK-1 NDK-1 PFK-1 SMG-1 PGK-1 VRK-1 SPK-1 PDHK-2 FEM-2 GSK-3 MEK-2 PMK-3 EGL-4 MIG-15 LET-502 LET-754 ZYG-8 B0001.4 C29F7.3 C14B9.8 C50D2.7 T05G5.5 E02H1.6 F38B2.4 F40F8.1 F46F11.1 F46H5.3 K02B2.1 T10B11.2 ZC434.8 ZK673.2
kinases
400 200 0
AAK-1
AAK-2
AKT-2
PLK-2

Fig. S5. Mitochondrial fusion-deficient worms (*fzo-1)* displayed increased protein abundance of cytosolic compensatory pathways (related to Fig. 3). Percentage difference in cytosolic protein abundance of critical intracellular compensatory pathways including: (A) protein synthesis, (B) proteostasis, (C) ubiquitin proteasome system (UPS), (D) autophagy, (E) cell death and (F) kinases determined by comparison between WT and *fzo-1(tm1133)* worms that were or were not submitted to long-term exercise [starting at the onset of adulthood (day 1), according to the protocol described in Figure 2D]. Proteomics was performed at day 10. Data are presented as mean ± SEM. *p <0.05 and **p <0.001 vs. WT Ctr. Detailed statistical analyses, number of biological replicates and sample size are described in *SI Appendix*, Table S3.

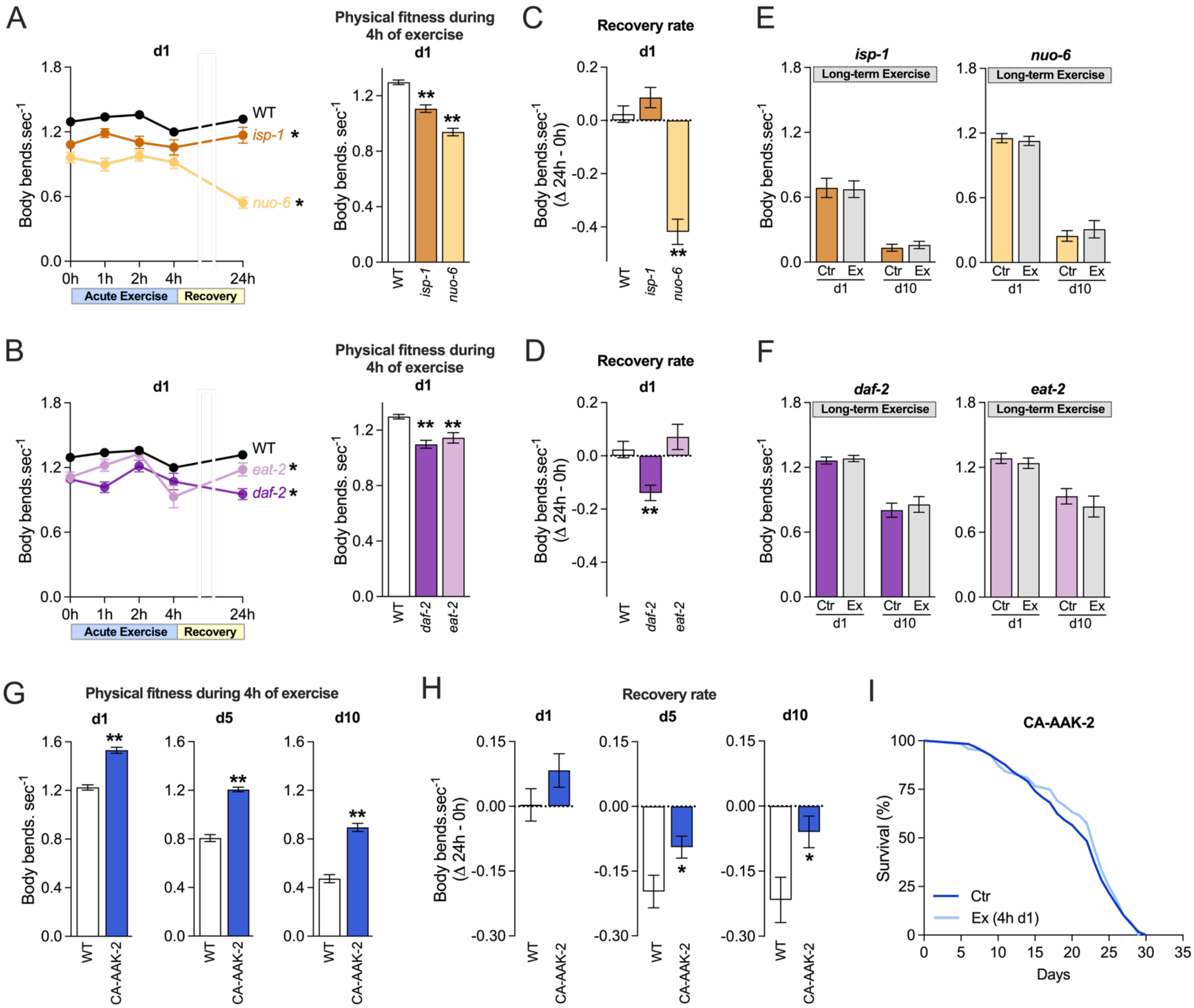


Fig. S6. Long-lived mutants *isp-1*, *nuo-6*, *daf-2* and *eat-2* have decreased physical fitness and exhibit no beneficial effects of exercise (related to Fig. 4). (A, B) Physical fitness during 4h of exercise and (C, D) recovery rate of WT, *isp-1(qm150), nuo-6(qm200), daf-2(e1370)* and *eat-2(ad1116)* worms submitted to acute exercise on day 1 of adulthood. (E, F) Physical fitness of *isp-1(qm150), nuo-6(qm200), daf-2(e1370)* and *eat-2(ad1116)* worms submitted to long-term exercise. (G) Physical fitness during 4h of exercise and (H) recovery rate of WT and CA-AAK-2 worms submitted to acute exercise on days 1, 5 and 10 of adulthood. (I) Lifespan of CA-AAK-2 worms submitted to 1 hour of acute exercise on day 1 of adulthood. Data are presented as mean ± SEM. *p <0.05 and **p <0.001 vs. WT. Detailed statistical analyses, number of biological replicates and sample size are described in *SI Appendix*, Table S3.

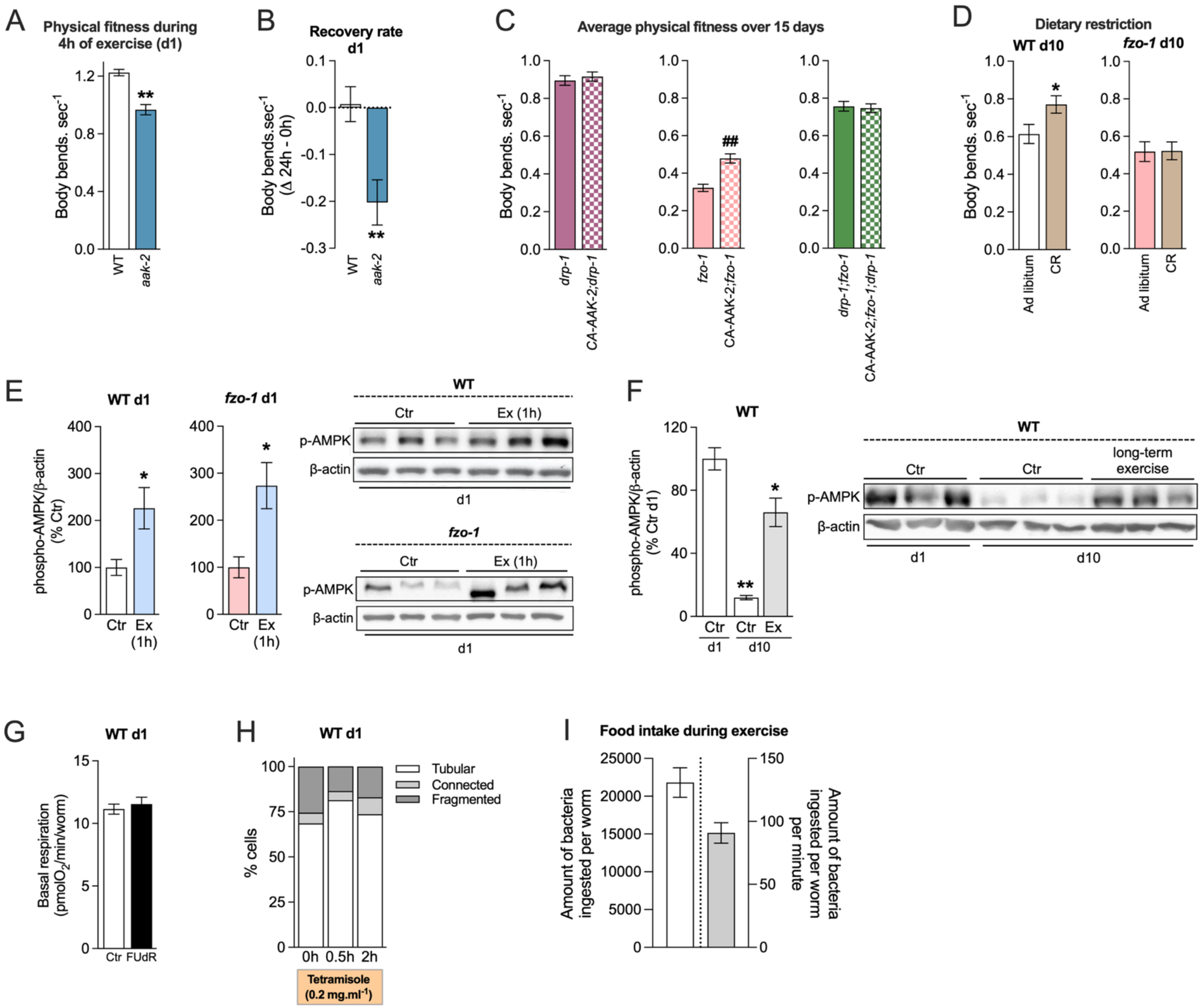


Fig. S7. AMPK is required for exercise-induced benefits (related to Fig. 5). (A) Physical fitness during 4h of exercise (average of 0h, 1h, 2h and 4h) –and (B) recovery rate of WT and *aak-2(gt33)* worms submitted to acute exercise on day 1 of adulthood. (C) Average physical fitness (average of d1, d5, d10 and d15) of mitochondrial dynamics mutants *drp-1(tm1108), fzo-1(tm1133)* and *fzo-1(tm1133);drp-1(tm1108)* in the absence or presence of CA-AAK-2 with aging. (D) Physical fitness of WT and *fzo-1(tm1133)* worms submitted to 10 days of dietary restriction, starting at the onset of adulthood (day 1). (E) Protein levels of phospho-AMPK of WT and *fzo-1(tm1133)* worms submitted to 1 hour of acute exercise on day 1 of adulthood. (F) Protein levels of phospho-AMPK of WT worms submitted to long-term exercise for 10 days, starting on day 1 of adulthood (according to the protocol described in Figure 2D). (G) Baseline O2 consumption of WT worms incubated with FUdR (20 mg.ml^{-1}) or vehicle for 4 hours in M9 buffer on day 1 of adulthood. (H) Mitochondrial morphology in body wall muscle cells of WT worms incubated with tetramisole (0.2 $mg.ml^{-1}$) on day 1 of adulthood. (I) Measurement of WT worms food ingestion during the exercise protocol period. This measurement was performed by counting the number of colony-forming units in the M9 buffer after 4 hours of swimming and compared with mock condition (no worms). Data are presented as mean ± SEM. *p <0.05 and **p <0.001 vs. WT, Ctr or Ad libitum and ##p <0.001 vs. *fzo-1(tm1133)*. Detailed statistical analyses, number of biological replicates and sample size are described in *SI Appendix*, Table S3.

Supplementary Tables

Table S2. *C. elegans* strains.

Strain	Genotype	Source
N2	wild type	Caenorhabditis Genetics Center
SJ4103	zcIs14[*myo-3*::GFP(mito)]	Caenorhabditis Genetics Center
CU6372	*drp-1(tm1108)* IV	Caenorhabditis Genetics Center
CU5991	*fzo-1(tm1133)* II	Caenorhabditis Genetics Center
MQ887	*isp-1(qm150)* IV	Caenorhabditis Genetics Center
MQ1333	*nuo-6(qm200)* I	Caenorhabditis Genetics Center
CB1370	*daf-2(e1370)* III	Caenorhabditis Genetics Center
DA1116	*eat-2(ad1116)* II	Caenorhabditis Genetics Center
WBM60	uthIs248[P*aak-2::aak-2*(genomic aa1-321)::GFP::unc-54 3'UTR+P*myo-2*::tdTOMATO]	Caenorhabditis Genetics Center
TG38	*aak-2(gt33)* X	Caenorhabditis Genetics Center
VC1024	*pdr-1(gk448)*	Caenorhabditis Genetics Center
	pink-1(tm1779)	BioResource Project
ZA20	*drp-1(tm1108)* IV*; eat-3(ad426)* II	Laboratory of Alexander M. van der Bliek
ZA21	N2 (P*myo3*::CFP::*Lgg-1* + P*myo3*::omYFP + *rol-6)*	Laboratory of Alexander M. van der Bliek
ZA22	*drp-1(tm1108)* IV; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)*	Laboratory of Alexander M. van der Bliek
ZA23	*fzo-1(tm1133)* II; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)*	Laboratory of Alexander M. van der Bliek
JVR122	bcIs78(P*myo-3*::mitoGFP (matrix GFP) + pRF4)	Laboratory of Jeremy M. Van Raamsdonk
JVR587	*drp-1* o/e: sybIs3765[P*eft-3*::*drp-1*+P*myo-3*::mCherry]	Laboratory of Jeremy M. Van Raamsdonk
JVR589	*fzo-1* o/e: sybIs3776[P*rpl-28*::*fzo-1*+P*myo-2*::mCherry]	Laboratory of Jeremy M. Van Raamsdonk
JVR622	*drp-1* o/e: sybIs3765[P*eft-3*::*drp-1*+P*myo-3*::mCherry]; bcIs78*(*p*myo-3*::mitoGFP (matrix GFP) + p*RF4)*	Laboratory of Jeremy M. Van Raamsdonk
JVR623	*fzo-1* o/e: sybIs3776[P*rpl-28*::*fzo-1*+P*myo-2*::mCherry]; bcIs78*(*p*myo-3*::mitoGFP (matrix GFP) + p*RF4)*	Laboratory of Jeremy M. Van Raamsdonk
AGD855 (11)	*sid-1(qt-9)* V*;* uthIs237[P*myo-3*::tomato::unc-54 3'UTR + P*myo3::sid-1*]	Laboratory of Malene Hansen
	syIs268[P*myo-3::tomm20*::mRFP]	Laboratory of S. Curran
WBM306 (8)	uthIs248[P*aak-2::aak*-2 genomic (aa1-321)::GFP::unc54 3'UTR, P*myo-2::*tdTOMATO*];* syIs268[P*myo-3::tomm20*::mRFP]	Laboratory of William B. Mair
WBM381 (8)	*drp-1(tm1108)* IV; syIs268[P*myo-3::tomm20*::mRFP]	Laboratory of William B. Mair
WBM382 (8)	*fzo-1(tm1133*) II; syIs268[P*myo-3::tomm20*::mRFP]	Laboratory of William B. Mair
WBM394 (8)	*drp-1(tm1108)* IV; uthIs248[P*aak-2::aak-2*(genomic aa1-321)::GFP::unc-54 3'UTR+P*myo-2*::tdTOMATO]	Laboratory of William B. Mair
WBM396 (8)	*fzo-1(tm1133)* II; uthIs248[P*aak-2::aak-2*(genomic aa1-321)::GFP::unc-54 3'UTR+P*myo-2*::tdTOMATO]	Laboratory of William B. Mair
WBM737 (8)	*fzo-1(tm1133)* II; *drp-1(tm1108)* IV; uthIs248[P*aak-2::aak-2*(genomic aa1-321)::GFP::unc-54 3'UTR+P*myo-2*::tdTOMATO]	Laboratory of William B. Mair
WBM884 (8)	*drp-1(tm1108)* IV; *fzo-1(tm1133)* II; hjIs37	Laboratory of William B. Mair

Table S3. Biological replicates, sample size and statistical analyses.

Figure (replicates)	Strain - group	Sample size	Statistical analyses	*p* value
1A (3)	zcls14 - d1	56		
	zcls14 - d5	67	two-tailed Student's t vs. d1	<.0001
	zcls14 - d10	66	two-tailed Student's t vs. d1	<.0001
	zcls14 - d15	45	two-tailed Student's t vs. d1	<.0001
1B (3)	zcls14 - d1	126		
	zcls14 - d5	173	chi-square vs. d1	<.0001
	zcls14 - d10	151	chi-square vs. d1	<.0001
1D (3)	zcls14 - d1 0h	126		
	zcls14 - d1 1h Exercise	138	chi-square vs. d1 0h	<.0001
	zcls14 - d1 2h Exercise	149	chi-square vs. d1 0h	<.0001
	zcls14 - d1 4h Exercise	173	chi-square vs. d1 0h	<.0001
	zcls14 - d1 24h Recovery	150	chi-square vs. d1 0h	0.0001
			chi-square vs. d1 4h	<.0001
1D (3)	zcls14 - d5 0h	156		
	zcls14 - d5 1h Exercise	159	chi-square vs. d5 0h	0.3565
	zcls14 - d5 2h Exercise	165	chi-square vs. d5 0h	0.0009
	zcls14 - d5 4h Exercise	154	chi-square vs. d5 0h	<.0001
	zcls14 - d5 24h Recovery	156	chi-square vs. d5 0h	0.6592
			chi-square vs. d5 4h	<.0001
1D (2)	zcls14 - d10 0h	135		
	zcls14 - d10 1h Exercise	74	chi-square vs. d10 0h	0.8980
	zcls14 - d10 2h Exercise	83	chi-square vs. d10 0h	0.9652
	zcls14 - d10 4h Exercise	86	chi-square vs. d10 0h	0.2928
	zcls14 - d10 24h Recovery	75	chi-square vs. d10 0h	0.1979
			chi-square vs. d10 4h	0.0135
1E (3)	zcls14 - d1 0h	56		
	zcls14 - d1 1h Exercise	55	two-tailed Student's t vs. d1 0h	0.1744
	zcls14 - d1 2h Exercise	53	two-tailed Student's t vs. d1 0h	0.2839
	zcls14 - d1 4h Exercise	56	two-tailed Student's t vs. d1 0h	0.0004
	zcls14 - d1 24h Recovery	37	two-tailed Student's t vs. d1 0h	0.7309
			two-tailed Student's t vs. d1 4h	0.0010
1E (3)	zcls14 - d5 0h	67		
	zcls14 - d5 1h Exercise	65	two-tailed Student's t vs. d5 0h	0.2880
	zcls14 - d5 2h Exercise	69	two-tailed Student's t vs. d5 0h	0.0062
	zcls14 - d5 4h Exercise	70	two-tailed Student's t vs. d5 0h	<.0001
	zcls14 - d5 24h Recovery	64	two-tailed Student's t vs. d5 0h	0.0444
			two-tailed Student's t vs. d5 4h	0.0002
1E (3)	zcls14 - d10 0h	66		
	zcls14 - d10 1h Exercise	66	two-tailed Student's t vs. d10 0h	0.0932
	zcls14 - d10 2h Exercise	68	two-tailed Student's t vs. d10 0h	<.0001
	zcls14 - d10 4h Exercise	70	two-tailed Student's t vs. d10 0h	<.0001

	zcIs14 - d10 24h Recovery	59	two-tailed Student's t vs. d10 0h	0.0372
			two-tailed Student's t vs. d10 4h	<.0001
1F (3)	zcIs14 - d1 Recovery rate (24h - 0h)	56		
	zcIs14 - d5 Recovery rate (24h - 0h)	67	two-tailed Student's t vs d1	0.0073
	zcIs14 - d10 Recovery rate (24h - 0h)	65	two-tailed Student's t vs d1	0.0050
1G (3)	zcIs14 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	5 (257)	Linear Regression	<.0001
	zcIs14 - d5 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	5 (335)		
	zcIs14 - d10 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	5 (329)		
2B (3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 0.0008
	N2 - d1, d5, d10, d15	67, 63, 54, 49		
	drp-1 - d1, d5, d10, d15	42, 48, 46, 48	Bonferroni post-hoc vs. N2	<.0001
	fzo-1 - d1, d5, d10, d15	42, 42, 39, 44	Bonferroni post-hoc vs. N2	<.0001
	drp-1;fzo-1 - d1, d5, d10, d15	69, 70, 70, 65	Bonferroni post-hoc vs. N2	<.0001
	drp-1;eat-3 - d1, d5, d10, d15	54, 48, 61, 57	Bonferroni post-hoc vs. N2	<.0001
2B (3)	N2 - d1+d5+d10+d15	233		
	drp-1 - d1+d5+d10+d15	184	two-tailed Student's t vs. N2	<.0001
	fzo-1 - d1+d5+d10+d15	167	two-tailed Student's t vs. N2	<.0001
	drp-1;fzo-1 - d1+d5+d10+d15	274	two-tailed Student's t vs. N2	<.0001
	drp-1;eat-3 - d1+d5+d10+d15	220	two-tailed Student's t vs. N2	<.0001
2C (3)	N2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	56, 55, 53, 56, 37		
	drp-1 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	56, 64, 64, 66, 62	two-tailed Student's t vs. N2 d1	0.0149
	fzo-1 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	51, 61, 61, 64, 51	two-tailed Student's t vs. N2 d1	<.0001
	drp-1;fzo-1 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	61, 65, 66, 69, 62	two-tailed Student's t vs. N2 d1	0.0145
	drp-1;eat-3 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	64, 65, 66, 64, 58	two-tailed Student's t vs. N2 d1	0.0388
2E (3)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 <.0001 <.0001
	zcIs14 - Control d1	67		
	zcIs14 - Exercise d1	68	Bonferroni post-hoc vs. Control d1	>.9999
	zcIs14 - Control d5	63		
	zcIs14 - Exercise d5	44	Bonferroni post-hoc vs. Control d5	>.9999
	zcIs14 - Control d10	54		
	zcIs14 - Exercise d10	65	Bonferroni post-hoc vs. Control d10	<.0001
	zcIs14 - Control d15	49		
	zcIs14 - Exercise d15	59	Bonferroni post-hoc vs. Control d15	<.0001
2E (3)	zcIs14 - Control (d1+d5+d10+d15)	233		
	zcIs14 - Exercise (d1+d5+d10+d15)	236	two-tailed Student's t vs. N2 d1	0.0008
2F (3)	zcIs14 - Control d5	173		
	zcIs14 - Exercise d5	147	chi-square vs. Control d5	0.0449
	zcIs14 - Control d10	151		
	zcIs14 - Exercise d10	130	chi-square vs. Control d10	0.0168
2G (3)	zcIs14 - Control d5, d10	2 (117)		
	zcIs14 - Exercise d5, d10	2 (109)		

2H (3)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.1408 0.8968
	drp-1 - Control d1	52		
	drp-1 - Exercise d1	53	Bonferroni post-hoc vs. Control d1	na
	drp-1 - Control d5	48		
	drp-1 - Exercise d5	43	Bonferroni post-hoc vs. Control d5	na
	drp-1 - Control d10	46		
	drp-1 - Exercise d10	43	Bonferroni post-hoc vs. Control d10	na
	drp-1 - Control d15	48		
	drp-1 - Exercise d15	48	Bonferroni post-hoc vs. Control d15	na
2H (3)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.1915 0.8630
	fzo-1 - Control d1	52		
	fzo-1 - Exercise d1	52	Bonferroni post-hoc vs. Control d1	na
	fzo-1 - Control d5	42		
	fzo-1 - Exercise d5	43	Bonferroni post-hoc vs. Control d5	na
	fzo-1 - Control d10	39		
	fzo-1 - Exercise d10	40	Bonferroni post-hoc vs. Control d10	na
	fzo-1 - Control d15	44		
	fzo-1 - Exercise d15	35	Bonferroni post-hoc vs. Control d15	na
2H (3)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.0058 0.3547
	drp-1;fzo-1 - Control d1	69		
	drp-1;fzo-1 - Exercise d1	69	Bonferroni post-hoc vs. Control d1	na
	drp-1;fzo-1 - Control d5	70		
	drp-1;fzo-1 - Exercise d5	65	Bonferroni post-hoc vs. Control d5	na
	drp-1;fzo-1 - Control d10	70		
	drp-1;fzo-1 - Exercise d10	63	Bonferroni post-hoc vs. Control d10	na
	drp-1;fzo-1 - Control d15	65		
	drp-1;fzo-1 - Exercise d15	75	Bonferroni post-hoc vs. Control d15	na
2H (3)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.0002 0.3151
	drp-1;eat-3 - Control d1	54		
	drp-1;eat-3 - Exercise d1	55	Bonferroni post-hoc vs. Control d1	na
	drp-1;eat-3 - Control d5	48		
	drp-1;eat-3 - Exercise d5	64	Bonferroni post-hoc vs. Control d5	na
	drp-1;eat-3 - Control d10	61		
	drp-1;eat-3 - Exercise d10	68	Bonferroni post-hoc vs. Control d10	na
	drp-1;eat-3 - Control d15	57		
	drp-1;eat-3 - Exercise d15	82	Bonferroni post-hoc vs. Control d15	na
2I (2)	*drp-1*;syIs268 - Control d10	35		
	drp-1;syIs268 - Exercise d10	45	chi-square vs. Control d10	0.6118

2I (2)	*fzo-1*;syIs268 - Control d10	50		
	fzo-1;syIs268 - Exercise d10	62	chi-square vs. Control d10	0.9552
2J (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.0207 0.0338
	*sid-1(qt-9);*uthIs237*;EV(RNAi)* - Control d1	24		
	*sid-1(qt-9);*uthIs237*;EV(RNAi)* - Exercise d1	24	Bonferroni post-hoc vs. Control d1	>.9999
	*sid-1(qt-9);*uthIs237*;EV(RNAi)* - Control d10	24		
	*sid-1(qt-9);*uthIs237*;EV(RNAi)* - Exercise d10	24	Bonferroni post-hoc vs. Control d10	0.0020
2J (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.8367 0.2014
	sid-1(qt-9);uthIs237;*drp-1*(RNAi) - Control d1	21		
	sid-1(qt-9);uthIs237;*drp-1*(RNAi) - Exercise d1	24	Bonferroni post-hoc vs. Control d1	na
	sid-1(qt-9);uthIs237;*drp-1*(RNAi) - Control d10	20		
	sid-1(qt-9);uthIs237;*drp-1*(RNAi) - Exercise d10	24	Bonferroni post-hoc vs. Control d10	na
2J (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.5419 0.9086
	*sid-1(qt-9);*uthIs237*;fzo-1(RNAi)* - Control d1	17		
	*sid-1(qt-9);*uthIs237*;fzo-1(RNAi)* - Exercise d1	24	Bonferroni post-hoc vs. Control d1	na
	*sid-1(qt-9);*uthIs237*;fzo-1(RNAi)* - Control d10	24		
	*sid-1(qt-9);*uthIs237*;fzo-1(RNAi)* - Exercise d10	24	Bonferroni post-hoc vs. Control d10	na
2J (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.6638 0.2271
	sid-1(qt-9);uthIs237;*eat-3*(RNAi) - Control d1	24		
	sid-1(qt-9);uthIs237;*eat-3*(RNAi) - Exercise d1	24	Bonferroni post-hoc vs. Control d1	na
	sid-1(qt-9);uthIs237;*eat-3*(RNAi) - Control d10	24		
	sid-1(qt-9);uthIs237;*eat-3*(RNAi) - Exercise d10	24	Bonferroni post-hoc vs. Control d10	na
4A (2)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 <.0001
	N2 - d1	47		
	isp-1 - d1	43	Bonferroni post-hoc vs. N2 d1	<.0001
	nuo-6 - d1	61	Bonferroni post-hoc vs. N2 d1	<.0001
	daf-2 - d1	57	Bonferroni post-hoc vs. N2 d1	0.1331
	eat-2 - d1	48	Bonferroni post-hoc vs. N2 d1	0.0868
	N2 - d10	42		
	isp-1 - d10	33	Bonferroni post-hoc vs. N2 d10	<.0001
	nuo-6 - d10	33	Bonferroni post-hoc vs. N2 d10	<.0001
	daf-2 - d10	34	Bonferroni post-hoc vs. N2 d10	0.0201
	eat-2 - d10	31	Bonferroni post-hoc vs. N2 d10	>.9999
4B (2)	N2 - d1, d5, d10, d15	38, 41, 42, 37		
	CA-AAK-2 - d1, d5, d10, d15	49, 37, 47, 50	two-tailed Student's t vs. N2	0.0261
	N2 - d1+d5+d10+d15	158		

4B (2)	CA-AAK-2 - d1+d5+d10+d15	183	two-tailed Student's t vs. N2	<.0001
4C (2)	N2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	38, 37, 36, 38, 37		
	CA-AAK-2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	40, 39, 40, 40, 45	two-tailed Student's t vs. N2 d1	0.0094
4C (2)	N2 - d5 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	45, 44, 47, 46, 42		
	CA-AAK-2 - d5 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	45, 45, 44, 45, 47	two-tailed Student's t vs. N2 d5	0.0013
4C (2)	N2 - d10 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	44, 41, 44, 45, 36		
	CA-AAK-2 - d10 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	44, 45, 46, 45, 43	two-tailed Student's t vs. N2 d10	0.0005
4D (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 <.0001 0.0005
	CA-AAK-2 - Control d1	49		
	CA-AAK-2 - Exercise d1	40	Bonferroni post-hoc vs. Control d1	>.9999
	CA-AAK-2 - Control d5	37		
	CA-AAK-2 - Exercise d5	47	Bonferroni post-hoc vs. Control d5	<.0001
	CA-AAK-2 - Control d10	47		
	CA-AAK-2 - Exercise d10	40	Bonferroni post-hoc vs. Control d10	<.0001
	CA-AAK-2 - Control d15	50		
	CA-AAK-2 - Exercise d15	50	Bonferroni post-hoc vs. Control d15	0.0181
4E (2)	CA-AAK-2;syIs268 – Control d10	49		
	CA-AAK-2;syIs268– Exercise d10	48	chi-square vs. Control d10	0.8374
5A (2)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 0.0033
	N2 - d1, d5, d10, d15	47, 41, 42, 37		
	aak-2 - d1, d5, d10, d15	45, 52, 44, 29	Bonferroni post-hoc vs. N2 d5	<.0001
5A (2)	N2 - d1+d5+d10+d15	167		
	aak-2 - d1+d5+d10+d15	170	two-tailed Student's t vs. N2	0.0010
5B (2)	N2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	38, 37, 36, 38, 40		
	aak-2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	38, 39, 39, 38, 45	two-tailed Student's t vs. N2 d1	0.0035
5C (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 <.0001 <.0001
	aak-2 - Control d1	45		
	aak-2 - Exercise d1	45	Bonferroni post-hoc vs. Control d1	>.9999
	aak-2 - Control d5	52		
	aak-2 - Exercise d5	41	Bonferroni post-hoc vs. Control d5	>.9999
	aak-2 - Control d10	44		
	aak-2 - Exercise d10	39	Bonferroni post-hoc vs. Control d10	<.0001
	aak-2 - Control d15	29		
	aak-2 - Exercise d15	44	Bonferroni post-hoc vs. Control d15	<.0001
5D (3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 <.0001
	N2 - d1, d5, d10, d15	62, 61, 56, 61		
	CA-AAK-2 - d1, d5, d10, d15	63, 84, 69, 71	Bonferroni post-hoc vs. N2	<.0001

	CA-AAK-2;*drp-1* - d1, d5, d10, d15	70, 90, 68, 69	Bonferroni post-hoc vs. N2	<.0001
			Bonferroni post-hoc vs. CA-AAK-2	<.0001
	CA-AAK-2;*fzo-1(tm1103)* - d1, d5, d10, d15	82, 72, 66, 65	Bonferroni post-hoc vs. N2	<.0001
			Bonferroni post-hoc vs. CA-AAK-2	<.0001
	CA-AAK-2;*drp-1;fzo-1* - d1, d5, d10, d15	57, 60, 60, 60	Bonferroni post-hoc vs. N2	<.0001
			Bonferroni post-hoc vs. CA-AAK-2	<.0001
5D (3)	N2 - d1+d5+d10+d15	240		
	CA-AAK-2 - d1+d5+d10+d15	287	two-tailed Student's t vs. N2	<.0001
	CA-AAK-2;*drp-1* - d1+d5+d10+d15	297	two-tailed Student's t vs. N2	0.0992
			two-tailed Student's t vs. CA-AAK-2	<.0001
	CA-AAK-2;*fzo-1(tm1103)* - d1+d5+d10+d15	285	two-tailed Student's t vs. N2	<.0001
			two-tailed Student's t vs. CA-AAK-2	<.0001
	CA-AAK-2;*drp-1;fzo-1* - d1+d5+d10+d15	237	two-tailed Student's t vs. N2	<.0001
			two-tailed Student's t vs. CA-AAK-2	<.0001
5E (3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 0.0146 0.1825
	drp-1 - d1, d5, d10, d15	56, 68, 64, 41		
	CA-AAK-2;drp-1 - d1, d5, d10, d15	64, 62, 66, 69	Bonferroni post-hoc vs. *drp-1*	na
5E (3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 <.0001
	fzo-1 - d1, d5, d10, d15	51, 57, 52, 50		
	CA-AAK-2;*fzo-1* - d1, d5, d10, d15	67, 72, 66, 65	Bonferroni post-hoc vs. f*zo-1* (d1)	<.0001
5E (3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 0.8906 <.0001
	drp-1;fzo-1 - d1, d5, d10, d15	61, 70, 56, 65		
	CA-AAK-2;*drp-1;fzo-1* - d1, d5, d10, d15	57, 60, 60, 60	Bonferroni post-hoc vs. *drp-1;fzo-1* d5 and d15	<.0011
S1A (2)	N2 - d1	20		
	N2 - d5	21	two-tailed Student's t vs. d1	0.6518
	N2 - d10	23	two-tailed Student's t vs. d1	<.0001
	N2 - d15	19	two-tailed Student's t vs. d1	<.0001
S1B (2)	zcIs14 - d1 0h	76		
	zcIs14 - d1 1h Starvation	74	chi-square vs. d1 0h	0.9681
	zcIs14 - d1 2h Starvation	83	chi-square vs. d1 0h	0.9504
	zcIs14 - d1 4h Starvation	103	chi-square vs. d1 0h	0.3712
S1C (3)	N2 – d1 0h	68		
	N2 – d1 1h Exercise	30	two-tailed Student's t vs. d1 0h	0.0001
S1D (3)	N2 – d1 0h	75		
	N2 – d1 1h Exercise	45	two-tailed Student's t vs. d1 0h	0.0485
S1E (2)	*hsp60-p*::GFP – d1 Control	20		
	hsp60-p::GFP – d1 4h Exercise	20	two-tailed Student's t vs. d1 Control	0.2237
S1E (2)	*hsp6-p*::GFP – d1 Control	26		
	hsp6-p::GFP – d1 4h Exercise	22	two-tailed Student's t vs. d1 Control	0.1495

S1G (3)	N2 – d1 Control	120		
	N2 – d1 1h Exercise	120	long-rank	0.1377
S1I (1)	N2 (P*myo3*::CFP::*lgg-1* + P*myo3*::omYFP + *rol-6)* – d1 Control	69		
	N2 (P*myo3*::CFP::*lgg-1* + P*myo3*::omYFP + *rol-6)* – d1 Chloroquine + 0h	57	two-tailed Student's t vs. Control	<.0001
	N2 (P*myo3*::CFP::*lgg-1* + P*myo3*::omYFP + *rol-6)* – d1 Chloroquine + 1h Exercise	20	two-tailed Student's t vs. Control	<.0001
			two-tailed Student's t vs. 0h	<.0001
	N2 (P*myo3*::CFP::*lgg-1* + P*myo3*::omYFP + *rol-6)* – d1 Chloroquine + 2h Exercise	50	two-tailed Student's t vs. Control	<.0001
			two-tailed Student's t vs. 0h	<.0001
	N2 (P*myo3*::CFP::*lgg-1* + P*myo3*::omYFP + *rol-6)* – d1 Chloroquine + 4h Exercise	49	two-tailed Student's t vs. Control	<.0001
			two-tailed Student's t vs. 0h	0.8787
S1J (2)	N2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	95		
	pdr-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	60	two-tailed Student's t vs. N2 d1	0.2690
	pink-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	60	two-tailed Student's t vs. N2 d1	0.7916
S2A (2)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 <.0001
	N2 - d1, d5, d10, d15	20, 21, 23, 19		
	drp-1 - d1, d5, d10, d15	20, 22, 24, 18	Bonferroni post-hoc vs. N2	0.0121
	fzo-1 - d1, d5, d10, d15	20, 22, 17, 11	Bonferroni post-hoc vs. N2	<.0001
S2A (2)	N2 - d1d5+d10+d15	83		
	drp-1 - d1+d5+d10+d15	84	two-tailed Student's t vs. N2	0.1265
	fzo-1 - d1+d5+d10+d15	70	two-tailed Student's t vs. N2	<.0001
S2B (2-3)			two-way ANOVA Time effect Strain effect Interaction	 <.0001 <.0001 0.0537
	N2 - d1, d5, d10, d15	81, 43, 68, 48		
	drp-1 o/e - d1, d5, d10, d15	39, 39, 40, 29	Bonferroni post-hoc vs. N2	na
	fzo-1 o/e - d1, d5, d10, d15	64, 61, 66, 43	Bonferroni post-hoc vs. N2	na
S2B (2-3)	N2 - d1+d5+d10+d15	240		
	drp-1 o/e - d1+d5+d10+d15	147	two-tailed Student's t vs. N2	<.0001
	fzo-1 o/e - d1+d5+d10+d15	234	two-tailed Student's t vs. N2	<.0001
S2C (3)	bcIs78 – d10	76		
	drp-1 o/e: sybIs3765;bcIs78 – d10	46	chi-square vs. WT	0.0591
	fzo-1 o/e: sybIs3776;bcIs78 – d10	70	chi-square vs. WT	0.4675
S2D (2)	*drp-1*; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 0h	28		
	drp-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 1h Exercise	19	chi-square vs. d1 0h	0.2777
	drp-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 2h Exercise	43	chi-square vs. d1 0h	0.6262
	drp-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 4h Exercise	32	chi-square vs. d1 0h	0.6235
S2D (2)	*fzo-1*; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 0h	28		
	fzo-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 1h Exercise	28	chi-square vs. d1 0h	0.2640
	fzo-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 2h Exercise	22	chi-square vs. d1 0h	0.3248

	fzo-1; (P*myo-3*::omYFP + P*myo-3*::mls::CFP + *rol-6)* – d1 4h Exercise	32	chi-square vs. d1 0h	0.2515
S2E (3)	N2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	220		
	drp-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	250	two-tailed Student's t vs. N2 d1	<.0001
	fzo-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	237	two-tailed Student's t vs. N2 d1	<.0001
	drp-1;fzo-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	261	two-tailed Student's t vs. N2 d1	<.0001
	drp-1;eat-3 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	259	two-tailed Student's t vs. N2 d1	0.0027
S2F (3)	N2 - d1 Recovery rate (24h - 0h)	56		
	drp-1 - d1 Recovery rate (24h - 0h)	56	two-tailed Student's t vs. N2 d1	0.0046
	fzo-1 - d1 Recovery rate (24h - 0h)	52	two-tailed Student's t vs. N2 d1	0.0470
	drp-1;fzo-1 - d1 Recovery rate (24h - 0h)	61	two-tailed Student's t vs. N2 d1	0.9375
	drp-1;eat-3 - d1 Recovery rate (24h - 0h)	65	two-tailed Student's t vs. N2 d1	0.1933
S2G (2)	zcIs14 - Control d10	40		
	zcIs14 - Exercise d10	40	two-tailed Student's t vs. zcIs14 - Ctr d10	0.0330
	drp-1 - Control d10	23		
	drp-1 - Exercise d10	16	two-tailed Student's t vs. *drp-1* - Ctr d10	0.2082
	fzo-1 - Control d10	16		
	fzo-1 - Exercise d10	20	two-tailed Student's t vs. *fzo-1* - Ctr d10	0.1760
S2H (1)	*sid-1(qt-9)*;uthIs237;*EV*(RNAi)	6		
	sid-1(qt-9);uthIs237;*drp-1*(RNAi)	3	two-tailed Student's t vs. *EV(RNAi)*	0.0446
S2H (1)	*sid-1(qt-9)*;uthIs237;*EV*(RNAi)	4		
	sid-1(qt-9);uthIs237;*hlh-1*(RNAi)	3	two-tailed Student's t vs. *EV(RNAi)*	0.0002
S2I (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.0255 0.0429
	sid-1(qt-9);EV(RNAi) - Control d1	17		
	sid-1(qt-9);EV(RNAi) - Exercise d1	20	Bonferroni post-hoc vs. Control d1	>.9999
	sid-1(qt-9);EV(RNAi) - Control d10	23		
	sid-1(qt-9);EV(RNAi) - Exercise d10	24	Bonferroni post-hoc vs. Control d10	0.0032
S2I (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.1424 0.0195
	sid-1(qt-9);drp-1(RNAi) - Control d1	16		
	sid-1(qt-9);drp-1(RNAi) - Exercise d1	16	Bonferroni post-hoc vs. Control d1	>.9999
	sid-1(qt-9);drp-1(RNAi) - Control d10	24		
	sid-1(qt-9);drp-1(RNAi) - Exercise d10	23	Bonferroni post-hoc vs. Control d10	0.0065
S6A (2)	N2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	37, 50, 50, 51, 42		
	isp-1 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	48, 47, 49, 49, 46	two-tailed Student's t vs. N2 d1	0.0013
	nuo-6 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	44, 44, 43, 45, 51	two-tailed Student's t vs. N2 d1	0.0036
S6A (2)	N2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	188		
	isp-1 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	193	two-tailed Student's t vs. N2 d1	<.0001
	nuo-6 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	176	two-tailed Student's t vs. N2 d1	<.0001
S6B (2)	N2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	37, 50, 50, 51, 42		
	daf-2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	47, 48, 47, 49, 45	two-tailed Student's t vs. N2 d1	0.0080

	eat-2 - d1 0h, 1h Ex, 2h Ex, 4h Ex, 24h Rec	34, 41, 36, 40, 33	two-tailed Student's t vs. N2 d1	0.0193
S6B (2)	N2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	188		
	daf-2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	191	two-tailed Student's t vs. N2 d1	<.0001
	eat-2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	151	two-tailed Student's t vs. N2 d1	0.0001
S6C (2)	N2 - d1 Recovery rate (24h - 0h)	37		
	isp-1 - d1 Recovery rate (24h - 0h)	48	two-tailed Student's t vs. N2 d1	0.2348
	nuo-6 - d1 Recovery rate (24h - 0h)	44	two-tailed Student's t vs. N2 d1	<.0001
S6D (2)	N2 - d1 Recovery rate (24h - 0h)	37		
	daf-2 - d1 Recovery rate (24h - 0h)	47	two-tailed Student's t vs. N2 d1	0.0002
	eat-2 - d1 Recovery rate (24h - 0h)	34	two-tailed Student's t vs. N2 d1	0.3982
S6E (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.9049 0.7337
	isp-1 - Control d1	21		
	isp-1 - Exercise d1	22	Bonferroni post-hoc vs. Control d1	na
	isp-1 - Control d10	33		
	isp-1 - Exercise d10	33	Bonferroni post-hoc vs. Control d10	na
S6E (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.7280 0.4012
	nuo-6 - Control d1	31		
	nuo-6 - Exercise d1	32	Bonferroni post-hoc vs. Control d1	na
	nuo-6 - Control d10	33		
	nuo-6 - Exercise d10	19	Bonferroni post-hoc vs. Control d10	na
S6F (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.5068 0.7514
	daf-2 - Control d1	29		
	daf-2 - Exercise d1	28	Bonferroni post-hoc vs. Control d1	na
	daf-2 - Control d10	34		
	daf-2 - Exercise d10	24	Bonferroni post-hoc vs. Control d10	na
S6F (2)			two-way ANOVA Time effect Exercise effect Interaction	 <.0001 0.2989 0.7067
	eat-2 - Control d1	29		
	eat-2 - Exercise d1	28	Bonferroni post-hoc vs. Control d1	na
	eat-2 - Control d10	31		
	eat-2 - Exercise d10	23	Bonferroni post-hoc vs. Control d10	na
S6G (2)	N2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	149		
	CA-AAK-2 - d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	159	two-tailed Student's t vs. N2 d1	<.0001
S6G (2)	N2 - d5 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	182		
	CA-AAK-2 - d5 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	179	two-tailed Student's t vs. N2 d5	<.0001
S6G (2)	N2 - d10 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	174		
	CA-AAK-2 - d10 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	180	two-tailed Student's t vs. N2 d10	<.0001
S6H (2)	N2 - d1 Recovery rate (24h - 0h)	38		
	CA-AAK-2 - d1 Recovery rate (24h - 0h)	40	two-tailed Student's t vs. N2 d1	0.1443

S6H (2)	N2 - d5 Recovery rate (24h - 0h)	45		
	CA-AAK-2 - d5 Recovery rate (24h - 0h)	45	two-tailed Student’s t vs. N2 d5	0.0264
S6H (2)	N2 - d10 Recovery rate (24h - 0h)	44		
	CA-AAK-2 - d10 Recovery rate (24h - 0h)	44	two-tailed Student’s t vs. N2 d10	0.0155
S6I (3)	CA-AAK-2 – Control	120		
	CA-AAK-2 – d1 1h Exercise	120	long-rank	0.4691
S7A (2)	N2 - Physical fitness d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	149		
	aak-2 - Physical fitness d1 (0h+ 1h Ex+ 2h Ex+ 4h Ex)	154	two-tailed Student’s t vs. N2 d1	<.0001
S7B (2)	N2 - d1 Recovery rate (24h - 0h)	38		
	aak-2 - d1 Recovery rate (24h - 0h)	38	two-tailed Student’s t vs. N2 d1	0.0010
S7C (3)	*drp-1* - d1+d5+d10+d15	229		
	CA-AAK-2;*drp-1* - D1+d5+d10+d15	261	two-tailed Student’s t vs. *drp-1*	0.5522
S7C (3)	*fzo-1* - d1+d5+d10+d15	210		
	CA-AAK-2;*fzo-1* - d1+d5+d10+d15	270	two-tailed Student’s t vs. *fzo-1*	<.0001
S7C (3)	*drp-1;fzo-1* - d1+d5+d10+d15	252		
	CA-AAK-2;*drp-1;fzo-1* - d1+d5+d10+d15	237	two-tailed Student’s t vs. *drp-1;fzo-1*	0.7733
S7D (3)	N2 – d10 AL	38		
	N2 – d10 CR	35	two-tailed Student’s t vs. N2 AL	0.0257
S7D (3)	*fzo-1* – d10 AL	33		
	fzo-1 – d10 CR	32	two-tailed Student’s t vs. *fzo-1* AL	0.9577
S7E (2)	N2 – phospho-AMPK/β-actin – d1	6		
	N2 – phospho-AMPK/β-actin – d1 1h Exercise	6	two-tailed Student’s t vs. N2 d1	0.0235
S7E (2)	*fzo-1* – phospho-AMPK/β-actin – d1	6		
	fzo-1 – phospho-AMPK/β-actin – d1 1h Exercise	6	two-tailed Student’s t vs. *fzo-1*d1	0.0090
S7F (3)	N2 – phospho-AMPK/β-actin – d1	3		
	N2 – phospho-AMPK/β-actin – d10	3	two-tailed Student’s t vs. N2 d1	0.0003
	N2 – phospho-AMPK/β-actin – d10 Exercise	3	two-tailed Student’s t vs. N2 d1	0.0418
			two-tailed Student’s t vs. N2 d10	0.0041
S7G (3)	N2 – d1 Control	9		
	N2 – d1 FUdR	9	two-tailed Student’s t vs. N2 Control	0.5513
S7H (1)	zcIs14 - d1 0h	55		
	zcIs14 - d1 0.5h Tetramisole	36	chi-square vs. d1 0h	0.6518
	zcIs14 - d1 2h Tetramisole	68	chi-square vs. d1 0h	0.3066

na = not applicable.

Table S4. Raw data of physical fitness decay of WT and the long-lived worms isp-1*(qm150)*, nuo-6*(qm200)*, daf-2*(e1370)* and eat-2*(ad1116)* with aging (Fig. 4*A*).

Body bends per second									
WT		**isp-1*(qm150)***		**nuo-6*(qm200)***		**daf-2*(e1370)***		**eat-2*(ad1116)***	
d1	**d10**	**d1**	**d10**	**d1**	**d10**	**d1**	**d10**	**d1**	**d10**
1.2167	1.3833	0.2500	0.4000	1.4667	0.0667	1.2000	1.1833	1.5500	1.0167
1.4000	0.8611	0.7167	0.1333	1.4167	0.3000	1.1667	1.0667	1.5000	1.2667
1.4333	1.1000	1.2333	0.1500	1.2667	0.1667	1.0500	0.6833	1.5167	0.7667
1.2500	1.1167	0.8000	0.0667	1.0833	0.2667	0.8333	0.1333	1.4667	1.3333
0.5000	0.8500	0.7333	0.2167	1.1333	0.0500	1.4833	0.2500	1.4333	1.1167
1.1167	0.8333	0.5667	0.1167	1.4667	0.1167	1.4000	1.1500	1.3333	1.2500
1.4500	0.1167	1.0667	0.0333	1.1500	0.5333	1.7000	0.9500	1.0333	1.0833
1.5500	0.9167	0.5333	0.4667	1.2333	0.0500	1.0833	1.2833	1.2167	1.2500
1.2667	1.2833	0.9000	0.0333	1.2333	0.7833	1.3333	1.2500	0.9167	1.2833
1.5833	0.1500	1.1833	0.3167	1.2333	0.0167	1.1333	1.1500	1.0167	1.1000
1.4667	0.6667	0.3333	0.0000	1.2167	0.9000	1.1500	0.8667	1.1000	1.2833
1.6333	1.0167	0.5667	0.2167	1.0000	0.2500	1.4167	1.3000	1.1000	1.1000
1.6667	0.8000	0.1833	0.7167	1.4000	0.1667	1.2333	0.9167	1.2500	1.1833
1.4667	1.0667	0.1167	0.0000	1.3000	0.0000	1.2167	0.3833	1.2833	1.4833
1.3667	1.0667	1.0833	0.0000	1.0333	0.0000	1.4833	0.4333	1.4667	1.2667
1.4500	1.0500	0.9000	0.0000	1.1333	0.0000	1.3333	0.4833	1.2500	0.7000
1.3667	1.0667	0.1500	0.0000	1.2333	0.0500	1.3000	0.3333	1.4500	0.9333
1.3000	1.0500	1.0000	0.2667	0.7167	0.0000	1.3833	0.7667	1.3500	1.0500
1.3667	1.1500	1.0833	0.0500	1.0833	0.0000	1.2833	0.3333	1.1500	0.8167
1.4000	1.3500	0.7500	0.0167	1.3167	0.0000	1.0833	0.6333	1.4000	0.7167
1.3000	0.9167	0.6500	0.0000	1.0000	0.8833	1.1167	1.1000	1.4000	1.2667
1.3000	1.1500	0.0500	0.0000	0.5000	0.5500	1.1000	1.1500	0.5833	1.1667
1.2833	0.8667	0.7167	0.0000	1.3500	0.7667	1.2667	0.8500	1.4333	0.0167
1.4167	0.8500	0.0167	0.0000	1.0000	0.1167	1.2500	1.0833	1.5500	1.0000
1.4833	0.5333	1.0167	0.2667	0.9333	0.4500	1.2167	0.6667	1.4833	0.7333
1.3333	0.8333	0.0333	0.0000	1.0833	0.3500	1.3667	0.3667	1.5500	0.0500
1.4500	0.9833	0.9667	0.0000	1.3667	0.1000	1.1333	1.1333	1.3667	0.1833
1.3667	0.9833	0.0167	0.1500	0.8500	0.6167	1.2667	1.0667	1.5000	0.8667
1.4167	1.1667	0.7000	0.1500	1.1000	0.0833	1.0667	0.1833	1.2833	0.0167
1.5833	1.0500	1.2333	0.0000	1.1333	0.3667	1.2333	0.2500	1.5500	0.9167
1.4333	1.2667	1.1333	0.0000	0.9833	0.0833	1.2500	0.8500	1.1833	0.7000
1.5000	1.1000	0.1000	0.0167	1.2833	0.0667	1.3667	0.5500	1.3333	
1.4000	1.5833	0.6000	0.6000	1.5667	0.0000	0.9667	1.1000	1.2500	
1.3667	1.3500	0.5667		1.3500		1.3667	1.4333	1.0667	
1.4833	1.2500	0.8167		0.8000		1.4333		1.2667	
1.2500	1.3000	0.7333		1.1167		1.4333		1.2833	
1.3667	1.2667	0.6667		1.4833		1.3500		1.4833	
1.4500	1.4000	0.9500		1.2167		1.3000		1.4667	
1.4167	1.2167	0.7333		1.0833		1.3333		0.5000	
1.2833	1.3833	0.4000		1.2833		1.1000		0.9167	
1.4667	0.2000	0.8000		1.5667		1.3500		1.4500	
1.5000	0.0000	0.7167		1.3500		1.3500		0.6000	
1.4500		1.4833		0.8000		1.1333		0.6167	
1.5000				1.1167		1.2833		1.2500	
1.4333				1.4833		1.2833		1.2333	
1.5333				1.2167		1.6667		1.0500	
1.4500				1.0833		1.5000		1.5333	
				1.0667		1.3333		1.3500	
				1.0667		1.4333			
				0.8830		1.2167			
				1.1167		1.3667			
				0.9333		1.2333			
				0.9333		1.1333			
				0.9000		1.1333			
				1.3500		1.1000			
				1.1833		1.3333			
				0.4000		1.5333			
				0.9000					
				0.9830					
				1.3500					
				1.0830					

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